

What is claimed is:

1.

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A method for amplifying a polynucleotide sequence comprising:
obtaining a linear, single strand polynucleotide sample;
ligating the ends of said sample to form a circular shaped sample;
introducing first and second sequence specific primers to said circular sample; and
initiating a primer extension amplification reaction to increase copy number of said circular sample.

2.

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The method of claim 1 wherein said step of obtaining a linear, single strand nucleic acid sample further comprises the steps of:
obtaining a sample of mRNA;
contacting said mRNA with reverse transcriptase without RNase H so that a first strand cDNA - mRNA complex is formed, and degrading said mRNA to form a polynucleotide sample.

3.

The method of claim 1 wherein said primer extension amplification reaction is a polymerase chain reaction.

4.

sub D1
The method of claim 1 wherein said polymerase chain reaction is employed with Taq polymerase or other heat-resisted DNA polymerase.

5.

The method of claim 1 wherein said PCR is touchdown PCR.

6.

sub C4
The method of claim 2 further comprising the step of: harvesting said amplified nucleotide product.

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D1

7.

The method of claim 1 wherein said ligase is T4 DNA ligase.

8.

The method of claim 1 wherein said primer is a degenerate primer.

9.

The method of claim 1 wherein said first and second primers are designed to hybridize to from about 4 to about 35 contiguous bases from a sequence known or suspected to be present in said nucleic acid sample.

10.

The method of claim 1 wherein said first primer comprises a 3' end of the same which is toward the 5' end of the nucleic acid sample.

11.

The method of claim 1 wherein one of said primers comprises a 3' end of the same which is toward the 3' end of said nucleic acid sample.

12.

A method for amplifying a nucleic acid molecule including the 5' and 3' ends comprising:
circularizing said nucleic acid molecule;
contacting said nucleic acid with first and second primers;
and
introducing a polymerase and a supply of nucleotide bases to said circularized nucleic acid molecule so that an amplification reaction occurs; wherein said region of said nucleic acid molecule outside of said first and second primers including the 3' and 5' ends of said molecule is amplified.

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SUB A1

Sub A/
Cont.

13.

The method of claim 1 wherein said ligase is T4 DNA ligase.

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D'

14.

The method of claim 1 wherein said primer is a degenerate primer.

15.

The method of claim 1 wherein said forward and reverse primers are designed to hybridize to from about 4 to about 35 contiguous bases from a sequence known or suspected to be present in said nucleic acid sample.

16.

The method of claim 1 wherein said one of said primers comprises a 3' end of the same which is toward the 5' end of the nucleic acid sample.

17.

The method of claim 1 wherein one of said primers comprises a 3' end of the same which is toward the 3' end of said nucleic acid sample.

18.

A method of cloning a full length cDNA sequence from an mRNA sample comprising:
obtaining a sample of mRNA;
transcribing said mRNA to cDNA in the absence of RNase H activity;
degrading said mRNA so that a single strand of cDNA is obtained;
ligating the ends of said cDNA;
selecting forward and reverse gene specific primers from known sequence of a gene suspected to be present in said cDNA; and
amplifying said cDNA by an extension chain reaction.

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19.

A method of sequencing a full length coding DNA or mRNA for a gene comprising;
obtaining a sample of mRNA;
transcribing said mRNA to cDNA in the absence of RNase H activity;
degrading said mRNA so that a single strand of cDNA is obtained;
ligating the ends of said cDNA;
selecting forward and reverse gene specific primers from known sequence of a gene suspected to be present in said cDNA;
amplifying said cDNA by a polymerase chain reaction; to obtain an amplified product and thereafter;
inserting said amplified product into a vector for sequencing.

20.

A set of nucleotide primers for use in PCR amplification of circularized cDNA comprising:
a forward primer of from about 4 to about 35 contiguous bases capable of hybridizing to a gene which is to be amplified, and
a reverse primer of from about 4 to about 35 contiguous bases capable of hybridizing to a gene which is to be amplified, wherein said forward primer is towards the 3' end of said gene and said reverse primer is towards the 5' end of said gene.

21.

A kit for amplifying first strand cDNA from a sample of mRNA comprising:
a DNA ligase,
a DNA polymerase,
a reverse transcriptase without RNase H activity;
an enzyme for degrading mRNA from a cDNA - mRNA hybrid;

Sub A2
cont.

~~A~~
each of the four deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP).

22.

A full length cDNA sequence said sequence determined by the method of claim 17.

~~A~~
23.

A cloned nucleic acid obtained by the method of claim 1.

24.

A method for amplifying a nucleic acid sequence comprising:
obtaining a linear, single strand nucleic acid sample;
ligating the ends of said sample to form a circular shaped sample;
introducing first and second sequence specific primers to said circular sample; wherein said sequence specific primers each have a 3' end directed toward the 5' or 3' end of said specific sequence, and
initiating an amplification reaction to amplify said circular sample.

25.

A method for amplifying a nucleic acid sequence comprising:
obtaining a linear, single strand nucleic acid sample;
ligating the ends of said sample to form a circular shaped sample;
introducing first and second sequence specific primers to said circular sample; wherein said sequence specific primers each have a 3' end directed toward the 5' or 3' end of said specific sequence, and
initiating a polymerase chain amplification reaction to amplify said circular sample.